Supplementary Information

Triterpenoids manipulate a broad range of virus-host fusion via wrapping the HR2 domain prevalent in viral envelopes

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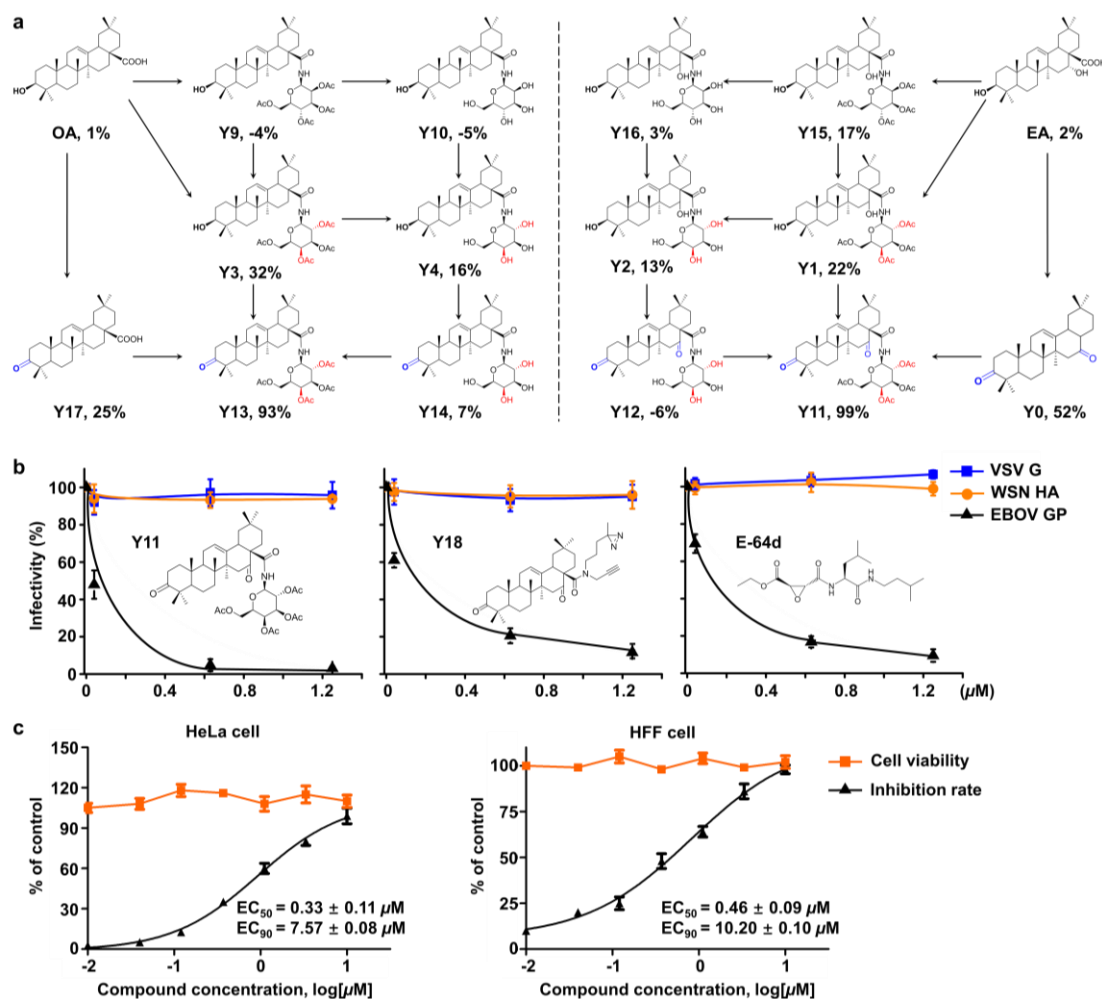**Supplementary Figure 19.** High resolution mass spectrum of **Y20**.

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Supplementary References

Supplementary Figure 1. Discovery of pentacyclic triterpene-glycoconjugates as EBOV entry inhibitors. (**a**) The structure-activity relationship (SAR) of triterpenoids and their derivatives as EBOV entry inhibitors. The potency of each compound was tested at a concentration of 1 μM by the EBOV pseudoparticle (EBOVpp) entry assay. The inhibition rate of the mock-treated cells was arbitrarily set to 0%. SAR analysis showed that conjugation with an acetylated galactose and oxidation of the 3' and 16'-hydroxyl groups on the triterpenoid backbone synergistically increased their inhibitory effect. (**b**) The dose-dependent effects of the triterpenoid lead **Y11**, the probe **Y18**, and the control compound E-64d on the entry of three pseudoviruses, EBOVpp, VSVpp, and IAVpp, which were tested in parallel. The data are presented as the mean $\pm$ standard deviation (s.d.) (n = 3). (**c**) Verification of the potency of the lead compound **Y11** against native Ebola virus (Makona) with MOI values of 1.5 and 20 for HeLa and HFF cells, respectively. The compound 3.47 served as the positive control with IC_{50} and IC_{90} values of 0.65 $\pm$0.13 and 6.20 $\pm$0.10 μM, respectively.
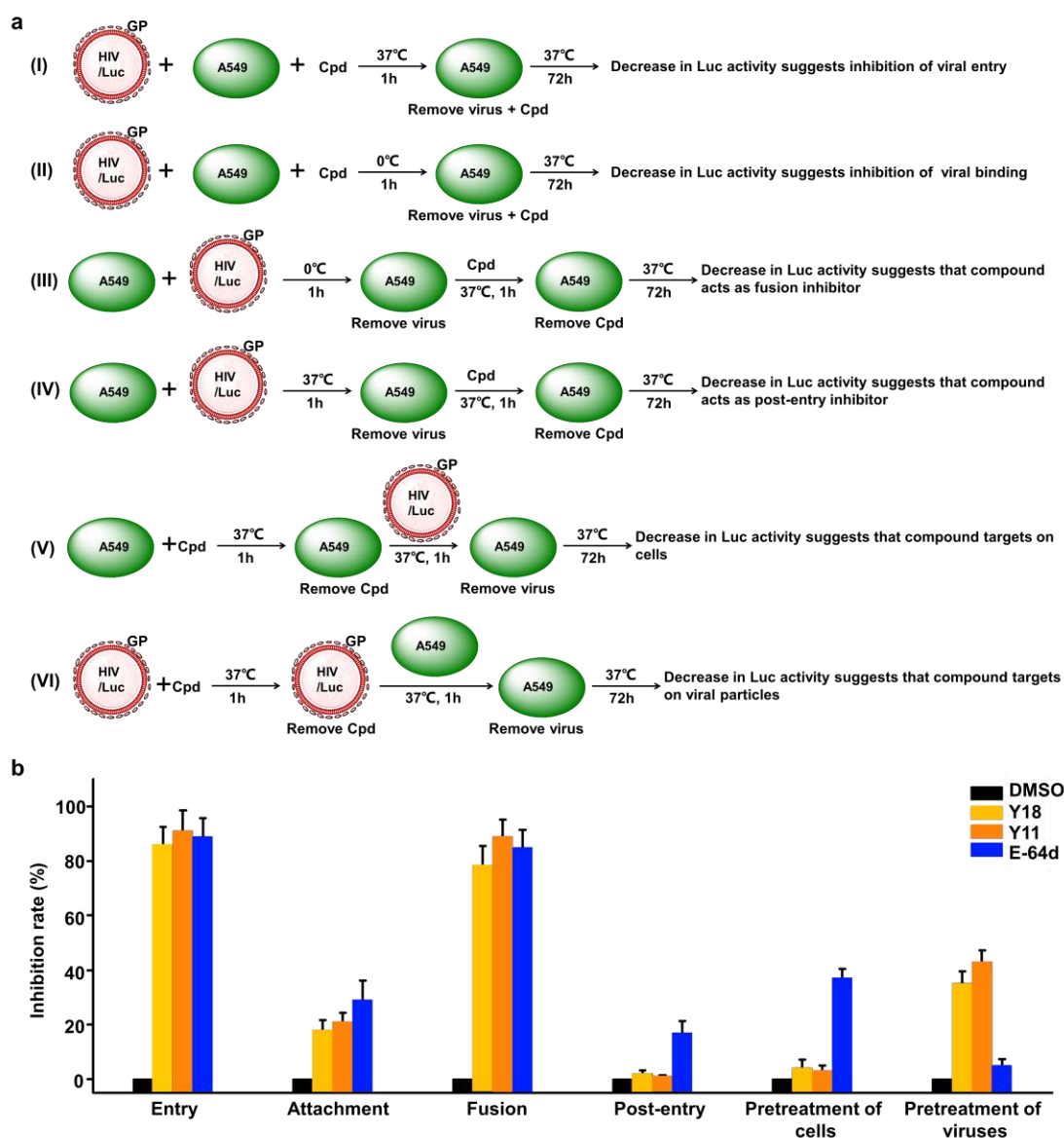
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In the fourth assay, post-entry experiment, cells were first treated with EBOVpp at 37 ℃ for 1 h to allow the virus entry into the cells. After being washed to remove the unbound viruses, the infected cells were treated with the lead compound at 37 ℃ for 72 h. In the fifth assay, pretreatment of cells, cells were incubated with the lead compound at 4 ℃ for 1 h and then washed to remove the unbound compounds. The pretreated cells were exposed to EBOVpp for 1 h, then washed to remove unbound viruses and cultured at 37 ℃ for 72 h. In the sixth assay, pretreatment of the viruses, EBOVpp was incubated with the lead compound at 4 ℃ for 30 minutes and then washed to remove the unbound compounds by ultrafiltration. The host cells were inoculated with the pretreated viruses for 1 h, then washed to remove unbound viruses and cultured at 37 ℃ for 72 h. The luciferase activity was tested to reflect the viral infectivity. (**b**) The inhibition rates of the lead compounds toward viral infection under different conditions. E-64d was used as a control. Data are presented as the mean ± s.d. (n = 3).
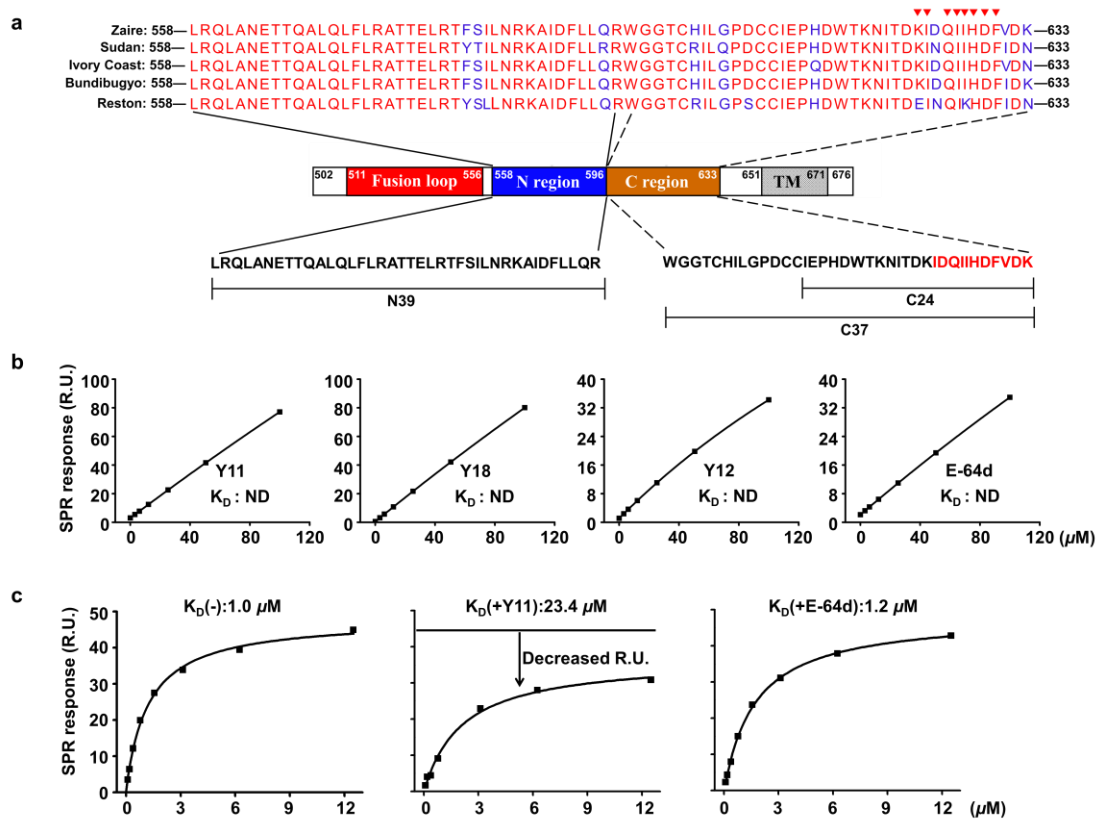
Supplementary Figure 3. Characterization of the affinity of triterpenoid compounds to N-terminal HR1, C-terminal HR2 and their effect on HR1-HR2 interaction. (**a**) Schematic representation of the primary EBOV GP2 structure, including a fusion loop (red), the N- (blue) and C-terminals (orange), and the transmembrane domain (TM, gray). The sequences of the N- and C-terminal of various EBOV subtypes, including the Zaire (strain Mayinga 1976), Sudan (strain Gulu), Bundibugyo (strain Uganda 2007), Ivory Coast (strain Cote d'Ivoire 1994), and Reston (strain Siena 1992) subtypes, are depicted and aligned. The conserved residues are highlighted in red, and the non-conserved residues are indicated in blue. The seven key interacting residues (K622, I623, Q625, I626, I627, D629 and F630) were conserved among the five different Ebola virus subtypes, although K622 and I627 were replaced with E and K, respectively, in the Reston subtype (red arrow). (**b**) SPR characterization of the affinity of the triterpenoid compounds to the HR1 peptide (EboIZN39IQ) immobilized on a chip; E-64d served as a control. No affinity was found for either compound. (**c**) SPR characterization of the effects of the triterpenoid compounds on HR1-HR2 affinity. The eboC24 peptide was allowed to flow across the surface of the eboIZN39IQ chip in the absence (K_D 1.0 μM) or presence of the lead compound **Y11** (K_D 23.4 μM); E-64d served as a control (K_D 1.2 μM).
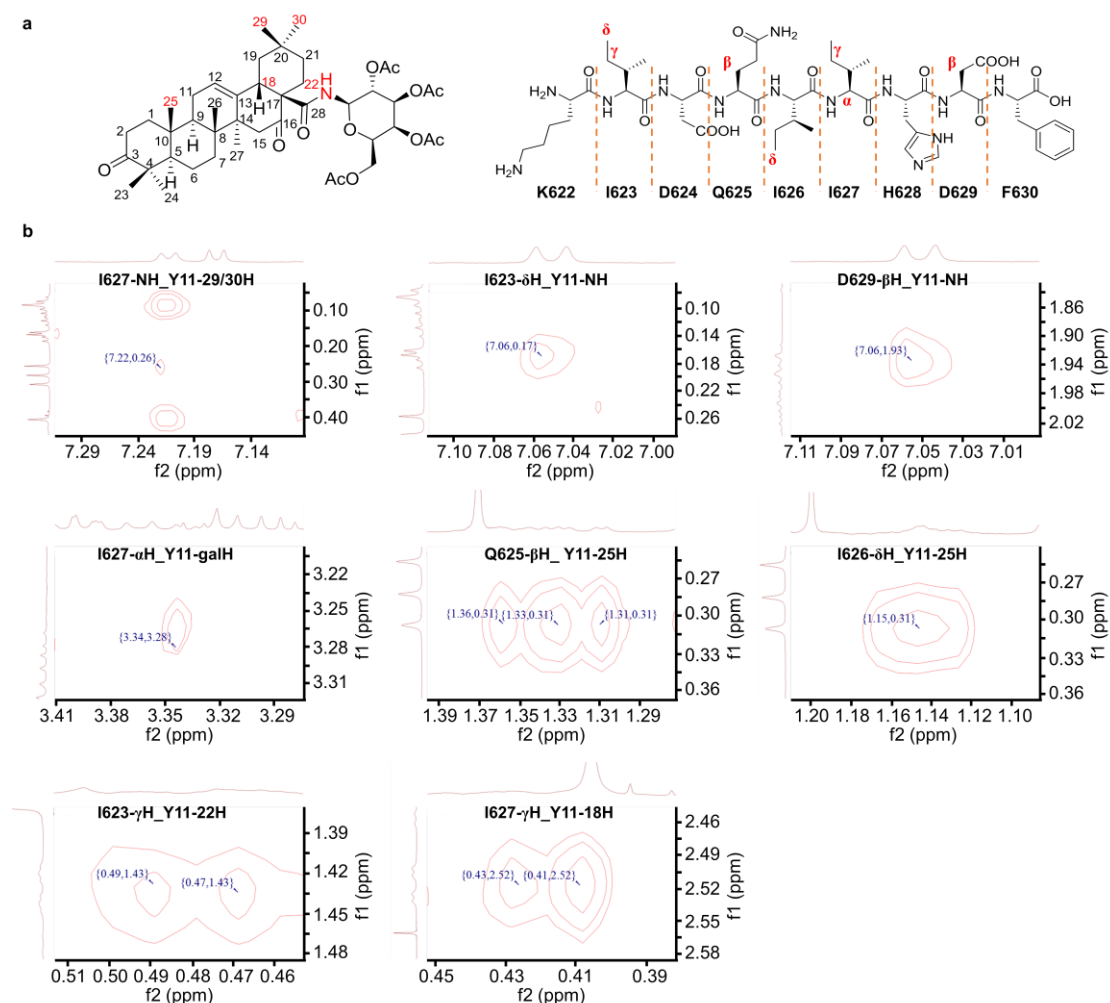
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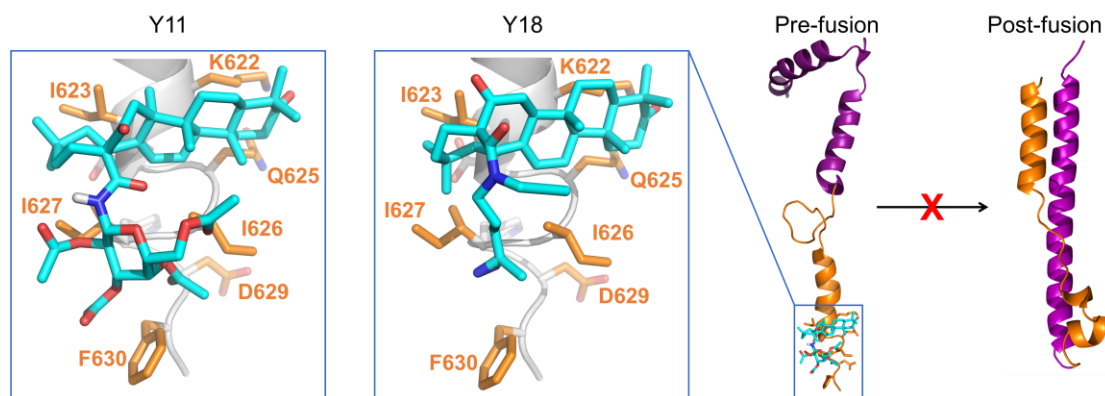
Supplementary Figure 5. Structural representations of Y11 and Y18 binding to the HR2 domain of EBOV GP (Protein Data Bank: 5JQ3), both according to the docking simulation. The amino acid residues that are involved in interactions with the lead compounds are labeled. The 3-keto group of **Y18** forms a hydrogen bond with K622, and the diazirine group of **Y18** is located near the crosslinking site I627.

a

HIV-HR1 (*IQN48*): *RMKQIEDKIEEIESKQKKIENEIARIKKL* IEAQQHLLQLTVWGIKQLQARILAVERYLKDQQLLGIWGCSGKLICTT

HIV-HR2 (C51): WNNMTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF
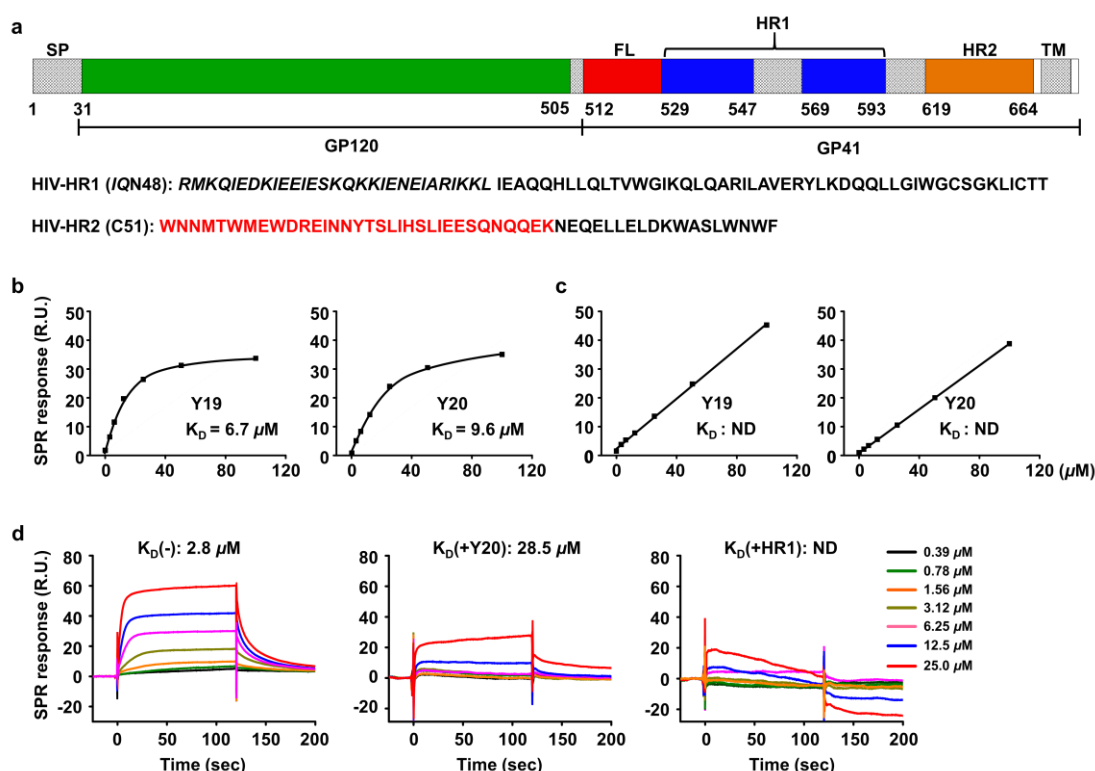
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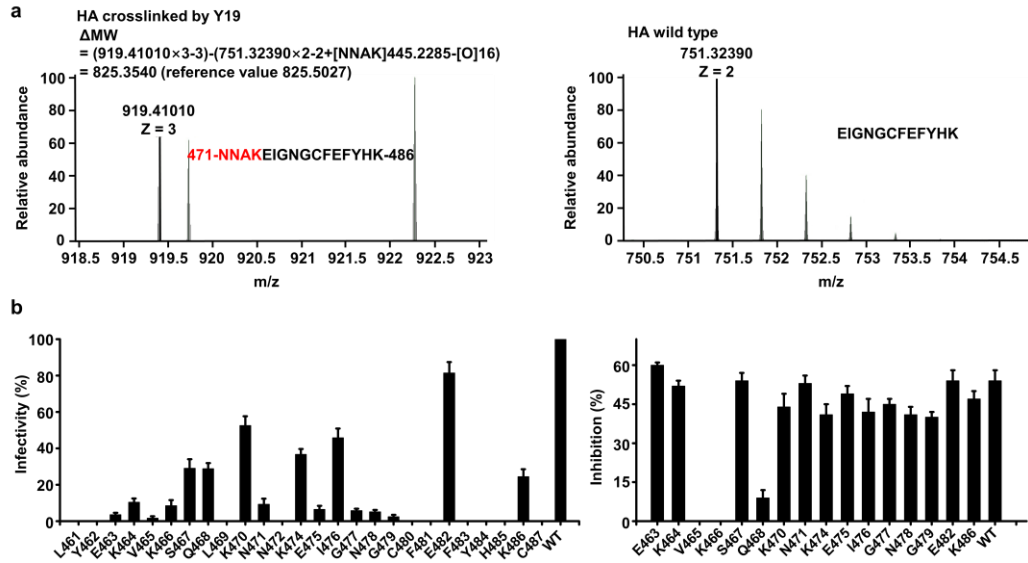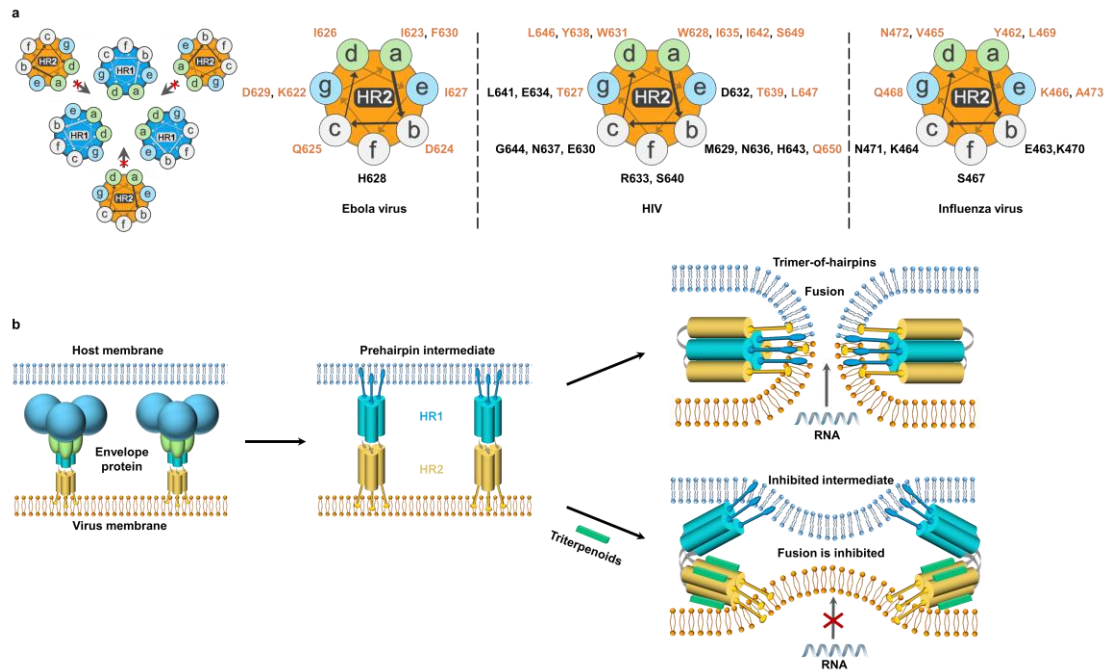
Supplementary Figure 7. Identification of HR2 in influenza HA2 as the domain targeted by the triterpenoid leads. (**a**) Identification of HR2 as the photocrosslinked domain by mass spectrometry. The influenza HA protein was photoaffinity labeled with the **Y21** probe and then analyzed by peptide mapping. A molecular weight increase of 825.5027, corresponding to photoactivated **Y21**, was observed in the peptide 471-NNAKEIGNGCFEFYHK-486 (influenza A/California/04/2009 (H1N1)). (**b**) Mapping of HR2 residues to elucidate their effects on viral infectivity and the potencies of the tested compounds. The mutation of K464, V465, K466 or Q468 to alanine significantly decreased the susceptibility of IAVpp to **Y3** (tested at 5 μM), disclosing the involvement of these residues in interactions with the triterpenoid compound. Mutating other residues to alanine had no such effects.

Supplementary Figure 8. The similar structural characteristics of HR2 in viruses provide the common structural basis by which triterpenoids block virus-cell membrane fusion. (**a**) A common helical wheel representation of the six-helix bundle, the post-fusion form of viral fusion proteins, and helical wheel representations of the HR2 domains of EBOV GP2, HIV GP41, and IAV HA with the amino acid residues identified. The residues at positions a and d are hydrophobic residues, whereas the residues at positions b, c, e, f, and g are polar residues. The residues at positions a and d of the HR1 domain form the trimer interface, and the HR2 domain folds into the groove of the HR1 trimer via its hydrophobic face formed by the a and d residues. The residues potentially involved in triterpenoid binding (depicted in orange) are primarily distributed at positions a, d, e and g. (**b**) A common mechanism for the triterpenoid-mediated inhibition of membrane fusion between cells and the Ebola, HIV, or influenza virus. The triterpenoids bind to the HR2 domain of a viral fusion protein, disrupting its interaction with the HR1 trimer. Thus, membrane fusion between the virus and the host cell is inhibited.
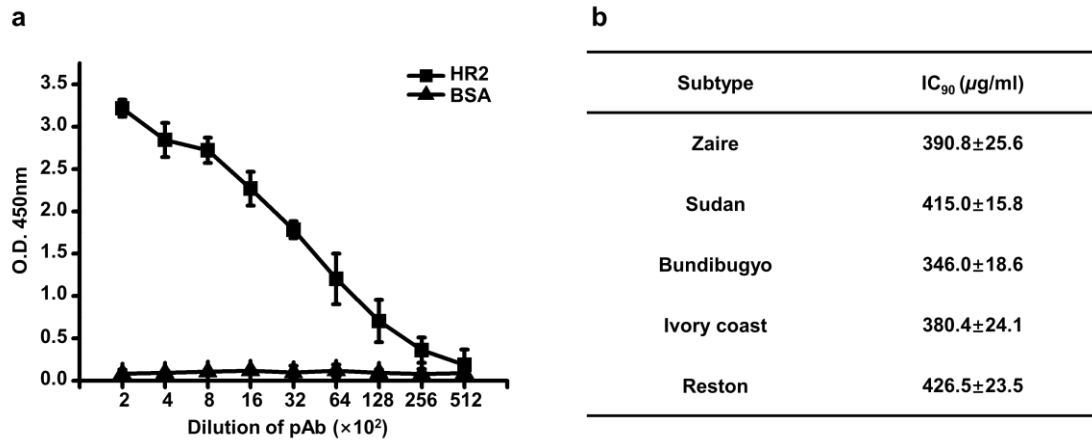
Supplementary Figure 9. Production and characterization of the HR2 peptide (KIDQIIHDF)-specific polyclonal antibody. (a) Characterization of the HR2 peptide-induced polyclonal antibody (pAb) by the ELISA method. Four hundred nanograms of the HR2 peptide was coated on the plate wells, and BSA was used as a control. The pAb was initially adjusted to a concentration of 1 mg/mL and then two-fold serially diluted. The first ($2^1 \times 10^2$ (n = 1)) and last ($2^9 \times 10^2$ (n = 9)) dilutions are shown. OD was measured at 450 nm. The experiment was repeated three times, and the results are presented as the mean $\pm$ s.d. (n = 3). **(b)** Characterization of the antiviral spectra of the HR2-induced antibody against various EBOV subtypes, including the Zaire (strain Mayinga 1976), Sudan (strain Gulu), Bundibugyo (strain Uganda 2007), Ivory Coast (strain Cote d'Ivoire 1994), and Reston (strain Siena 1992) subtypes. The IC_{90} values are presented as the mean $\pm$ s.d. (n = 3).
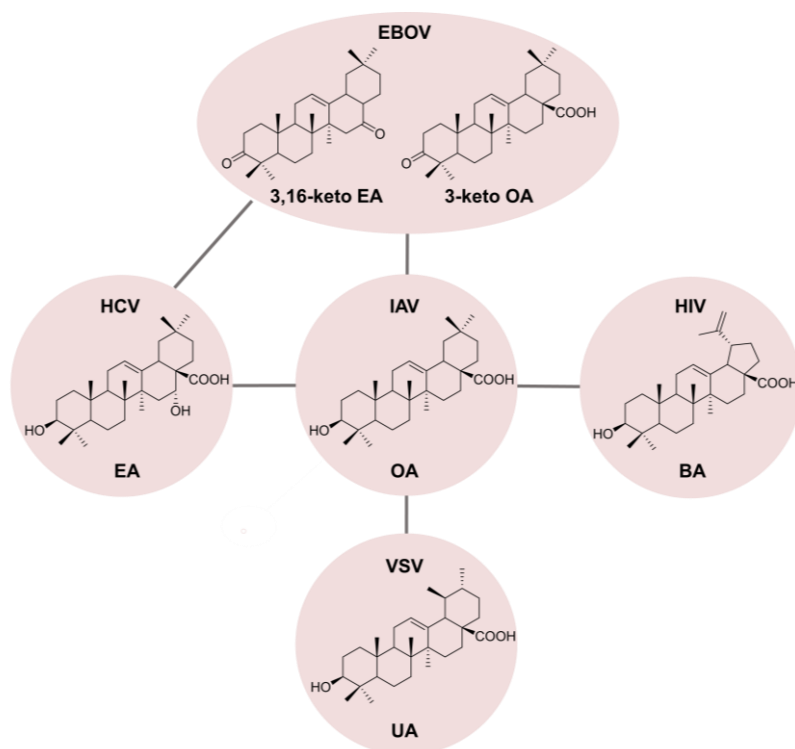
Supplementary Figure 10. The structure-activity relationship of triterpenoids against viruses according to our study. According to our study, although triterpenoids contain the alike pentacyclic scaffolds, many small differences appear in the structures of different types of triterpenoids, leading to their specific inhibition of different viruses: EA and its derivatives tend to specifically inhibit HCV fusion; OA and its derivatives tend to specifically inhibit influenza virus fusion; BA and its derivatives tend to specifically inhibit HIV fusion; the oxidation products of EA and OA and their derivatives tend to specifically inhibit EBOV fusion; and ursolic acid and its derivatives tend to specifically inhibit VSV fusion.

Supplementary Table 1. Broad antiviral spectra of the tested compounds against various EBOV subtypes

subtype	IC_{50} (nM) / IC_{90} (μM)				
	Zaire	Sudan	Budibugyo	Ivory Coast	Reston
Y11	59.2±1.6 / 0.29±0.01	48.9±4.1 / 0.24±0.02	56.2±5.8 / 0.28±0.03	40.6±3.2 / 0.20±0.12	57.2±5.6 / 0.29±0.06
Y18	467.3±10.2 / 1.86±0.04	383.8±9.0 / 1.53±0.13	481.4±9.9 / 1.93±0.14	390.1±13.8 / 1.56±0.16	173.1±7.9 / 0.69±0.11
Y12	ND	ND	ND	ND	ND
E-64d	77.4±5.6 / 0.69±0.03	96.1±8.9 / 0.78±0.12	106.8±6.5 / 1.28±0.22	88.4±6.1 / 0.98±0.08	119.7±5.9 / 1.02±0.22

The IC_{50} and IC_{90} values are presented as the mean $\pm$ s.d. (n = 3). ND, no data due to no activity was detected.

Supplementary Table 2. Mapping of the constitutive residues of HR2 to elucidate their effects on viral infectivity and the potencies of the tested compounds

Variant	IC$_{50}$ (nM)			Variant	IC$_{50}$ (nM)		
	E-64d	Y11	Y18		E-64d	Y11	Y18
E611A	91.2±5.6	49.3±3.9	388.8±8.6	T620A	88.5±5.7	72.9±9.6	145.1±1.6
P612A	110.9±4.1	91.5±2.4	329.6±6.6	D621A	93.2±1.2	67.3±2.6	727.9±3.6
H613A	97.0±3.2	56.8±1.2	393.9±7.6	V631A	94.3±3.4	74.7±5.0	472.3±7.0
D614A	78.9±3.1	69.2±2.5	512.1±8.7	D632A	87.5±4.1	84.2±3.0	495.2±7.1
W615A	88.8±5.1	80.2±3.6	406.9±9.5	K633A	56.6±2.6	69.5±6.8	712.5±7.9
T616A	79.5±6.4	64.1±5.9	449.9±7.9	T634A	78.2±5.8	68.9±3.1	527.0±5.9
K617A	60.6±7.2	46.5±5.8	304.1±8.6	L635A	94.1±7.6	52.8±5.0	669.1±5.8
N618A	83.4±2.8	41.4±3.6	362.7±9.6	P636A	34.9±5.3	69.4±2.9	459.8±6.1
I619A	78.1±3.6	62.2±5.1	276.5±8.6	D637A	69.1±8.1	71.2±6.6	478.9±6.9
wild type	77.4±5.6	59.2±1.6	467.3±10.2	wild type	77.4±5.6	59.2±1.6	467.3±10.2

The IC$_{50}$ values are presented as the mean ±s.d. (n = 3).

Supplementary methods

General materials and methods

NMR spectra were recorded on a Bruker DRX 400 spectrometer at ambient temperature. ^{1}H NMR chemical shifts were referenced to the internal standard TMS (δ_H = 0.00) or the solvent signal (δ_H = 3.31 for the central line of MeOD). ^{13}C NMR chemical shifts are referenced to the solvent signal (δ_C = 77.00 for the central line of CDCl$_3$, δ_C = 49.00 for the central line of MeOD). High-resolution mass spectra (HRMS) were obtained with an APEX IV FT_MS (7.0 T) spectrometer (Bruker) in positive ESI mode. Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated silica gel 60 F$_{254}$ plate (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detected by staining with a yellow solution containing Ce(NH$_4$)$_2$(NO$_3$)$_6$ (0.5 g) and (NH$_4$)$_6$Mo$_7$O$_{24}$ 4H$_2$O (24.0 g) in 6% H$_2$SO$_4$ (500 mL), followed by heating. Flash column chromatography was performed on silica gel 60 (200−300 mesh, Qingdao Haiyang Chemical Co. Ltd.).[1,2]

Synthesis of probe Y18 (**Supplementary Scheme 1**)

1. Synthesis of 3-(3-methyl-3*H*-diazirin-3-yl)propan-1-ol (**1**).

5-hydroxy-2-pentanone (10 g, 0.1 mol) was added to 40 mL of NH$_3$ and stirred for 3 h at -78 ℃. Hydroxylamine-O-sulfonic acid (15 g, 0.13 mol) was dissolved in methanol and poured into the reaction mixture. After overnight stirring, the white precipitate was filtered and methanol was added to the ice-cold reaction mixture, followed by triethylamine. Iodine was slowly added until the iodine colour persisted. After 2 h, the methanol was evaporated and the reaction mixture was extracted with ether and dried over MgSO$_4$. Vacuum distillation was performed to obtain a final pale yellow oil **1** (4.5 g, 40.3% overall yield).

2. Synthesis of 3-(3-methyl-3*H*-diazirin-3-yl)propyl 4-methylbenzenesulfonate (**2**).

Three millilitres of triethylamine was added to a solution of compound **1** (1.14 g, 10 mmol) in 40 mL of CH$_2$Cl$_2$ at 0 ℃, followed by step-wise addition of *p*-toluenesulfonyl chloride (2.86 g, 15 mmol). The reaction mixture was stirred at

room temperature (RT) overnight. After completion, as determined by thin layer chromatography (TLC), the reaction mixture was treated with 1 N HCl. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2. The combined extracts were washed with 1 N HCl and 1 N NaOH, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by column chromatography (petroleum ether:ethyl acetate (EtOAc), 10:1 v/v) to yield compound **2** as a viscous oil (2.2 g, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (s, 3H), 1.38-1.42 (m, 2H), 1.49-1.56 (m, 2H), 2.45 (s, 3H), 3.99 (t, 2H, J = 6.1 Hz), 7.36 (d, 2H, J = 8.0 Hz), 7.78 (d, 2H, J = 8.3 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ 19.6, 21.6, 23.5, 25.0, 30.2, 69.3, 127.8 (2C), 129.8 (2C), 132.9, 144.8.

3. Synthesis of N-(3-(3-methyl-3H-diazirin-3-yl)propyl)prop-2-yn-1-amine (**3**).

A reaction mixture of compound **2** (1.4 g, 5.22 mmol), propargylamine (1.4 mL, 20.9 mmol), and Na_2CO_3 (1.6 g, 15.1 mmol) was suspended in 20 mL of acetonitrile. The mixture was then refluxed at 50 ℃. After 2 days, the solvent was evaporated and the residue was suspended with water (30 mL). The product was extracted with CH_2Cl_2 and dried over $MgSO_4$. After solvent evaporation, the product was purified by column chromatography (CH_2Cl_2:methanol, 15:1 v/v) to yield compound **3** as a yellow oil (0.9 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (s, 3H), 1.34-1.44 (m, 5H), 2.23 (t, 1H, J = 2.4 Hz), 2.64-2.68 (m, 2H), 3.40 (d, 2H, J = 2.4 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ 19.6, 24.1, 25.5, 31.9, 37.9, 47.7, 71.2, 81.9.

4. Synthesis of (4aR,5R,6aS,6bR,10S,12aR)-5,10-dihydroxy-2,2,6a,6b,9,9,12a -heptamethyl-N-(3-(3-methyl-3H-diazirin-3-yl)propyl)-N-(prop-2-yn-1-yl)-1,3,4,5 ,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxam ide (**4**).

N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 50 mg, 0.25 mmol) was added to echinocystic acid (EA, 80 mg, 0.17 mmol) in 6 mL of dry tetrahydrofuran (THF) with stirring. After 0.5 h, compound **3** (30.2 mg, 0.2 mmol) was added to the mixture. The mixture was stirred at RT until completion, as determined by TLC. The solvent was evaporated, the residue was suspended in

EtAc (30 mL), washed with water (20 mL × 3) and saline, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (petroleum ether:EtOAc, 3:1 v/v) to yield compound **4** as a white solid (78 mg, 76% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 0.79, 0.81, 0.90, 0.91, 0.93, 0.99, 1.01, 1.31 (8 × CH_3), 0.73-2.07 (m, other aliphatic ring protons), 2.27 (brs, 1H), 3.13 (dd, 1H, J = 4.3, 13.8 Hz), 3.21 (dd, 1H, J = 4.0, 10.6 Hz), 3.29-3.43 (m, 1H), 4.06-4.10 (m, 1H), 4.21 (brs, 1H), 4.32-4.36 (m, 1H), 5.52 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5, 15.5, 17.0, 18.2, 19.7, 21.8, 23.3, 25.4, 26.4, 27.1, 28.0, 28.1, 29.3, 31.7, 32.6, 33.0, 34.7, 36.4, 37.0, 38.2, 38.5, 38.7, 39.8, 41.6, 42.7, 46.7, 46.9, 48.0, 50.4, 55.3, 72.2, 72.2, 78.8, 79.2, 122.8, 142.6, 175.5. ESI-HRMS (m/z) [M+H]$^+$ calcd for $C_{38}H_{60}N_3O_3$, 606.4629, found 606.4633.

5. Synthesis of (4aR,6aS,6bR,12aR)-2,2,6a,6b,9,9,12a-heptamethyl-N-(3 -(3-methyl-3H-diazirin-3-yl)propyl)-5,10-dioxo-N-(prop-2-yn-1-yl)-1,3,4,5,6,6a, 6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxamide (**Y18**).

Dess-Maritin periodinane (212 mg, 0.5 mmol) was added to a solution of compound **4** (60 mg, 0.1 mmol) in 8 mL of CH_2Cl_2. The reaction was stirred at RT for 30 min after completion (TLC), and then excess saturated aqueous $Na_2S_2O_3$ was added to the reaction mixture. The mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (petroleum ether : EtOAc, 4:1 v/v) to afford **Y18** as a white solid (50 mg, 80% yield). ^1H NMR (400 MHz, $CDCl_3$): δ 5.53 (brs, 1H), 4.03 (d, 1H, J = 18.4 Hz), 3.84 (d, 1H, J = 16.7 Hz), 3.65-3.72 (m, 1H), 3.21-3.35 (m, 2H), 2.72 (d, 1H, J = 12.8 Hz), 2.50-2.59 (m, 1H), 2.39-2.41 (m, 1H), 2.27 (s, 1H), 2.06-2.13 (m, 2H), 1.88-1.98 (m, 3H), 1.68 (td, 2H, J = 3.3, 12.9 Hz), 1.18, 1.09, 1.07, 1.04, 0.94, 0.92, 0.87 (s, 3H each, CH_3) (**Supplementary Fig. 11**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 217.31, 213.87, 170.19, 141.04, 123.34, 77.20, 73.09, 60.12, 55.18, 50.06, 49.25, 47.32, 46.74, 46.61, 46.13, 45.69, 39.71, 39.00, 36.78, 36.67, 34.24, 34.02, 32.48,

31.75, 30.42, 26.53, 26.19, 26.10, 25.41, 23.69, 23.48, 22.56, 21.42, 19.76, 19.48, 17.09, 15.07 (**Supplementary Fig. 12**). ESI-HRMS (m/z) [M+H]$^+$ calcd for $C_{38}H_{56}N_3O_3$, 602.4322, found 602.4322 (**Supplementary Fig. 13**).

Synthesis of Biotin-azide (**Supplementary Scheme 2**)

1. Synthesis of 2,5-dioxopyrrolidin-1-yl 5-((3aS, 4S, 6aR)-2-oxohexahydro-1H -thieno[3,4-d] imidazole-4-yl)pentanoate (**1**).

   To D-biotin (488 mg, 2 mmol) stirring in 8 mL DMF, N-hydroxysuccinimide (344 mg, 3 mmol) and EDC (576 mg, 3 mmol) were added. The mixture was stirred at rt overnight. The reaction mixture was then poured into ice (400 mL), and the precipitate was filtered. The precipitate was washed twice and dried under reduced pressure to afford **1** as a white solid (580 mg, 85%).

2. Synthesis of N-(2-azidoethyl)-5-((3aS, 4S, 6aR)-2-oxohexahydro-1H -thieno[3,4-d] imidazole-4-yl)pentanoate (**Biotin-azide**).

   To compound **1** (96mg, 0.28 mmol) stirring in 3 mL DMF, TEA (80 μL, 0.70 mmol) and 2-azidoethylamine (48 mg, 0.56 mmol) were added. The reaction was stirred for 6 h at rt. The contents were evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH/AcOH, 100/10/1 v/v/v) to give **Biotin-azide** as a white solid (46.4 mg, 53%). 1H NMR (400 MHz, d6-DMSO): δ4.30 (dd, 1H, J = 7.5, 4.9 Hz), 4.12 (dd, 1H, J = 7.7, 4.4 Hz), 3.33 (t, 2H, J = 5.8 Hz), 3.23 (t, 2H, J = 5.6 Hz), 3.09 (m, 1H), 2.82 (dd, 1H, J = 12.4, 5.1 Hz), 2.57 (d, 1H, J = 12.4 Hz), 2.08 (t, 2H, J = 7.4 Hz), 1.22-1.68 (m, 6H); 13C NMR (100 MHz, d6-DMSO): δ 180.01, 176.44, 61.02, 59.19, 55.38, 49.98, 38.13, 35.12, 28.17, 25.13.

Synthesis of Y0 (**Supplementary Scheme 3**)

1. Synthesis of benzyl(4aR,5R,6aS,6bR,10S,12aR)-5,10-dihydroxy-2,2,6a,6b, 9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahy dropicene-4a(2H)-carboxylate (**1**).

   K_2CO_3 (604 mg, 4.4 mmol) was added to a solution of echinocystic acid (EA, 1 g, 2.2 mmol) and benzyl bromide (562 mg, 3.3 mmol) in dimethylformamide (DMF; 30

mL). The resulting solution was stirred vigorously for 24 hours at 60 ℃. After removing DMF under a vacuum, the residue was purified by chromatography over silica gel to afford compound **1** as a white solid (1.1 g, 89% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.29-7.37 (m, 5H), 5.38 (t, 1H, J = 3.5 Hz), 5.01-5.08 (m, 2H), 4.55 (t, 1H, J =3.3 Hz), 3.21 (dd, 1H, J = 4.3, 11.6 Hz), 3.09 (dd, 1H, J = 4.4, 14.4 Hz), 2.12-2.19 (m, 1H), 1.34, 0.98, 0.96, 0.90, 0.89, 0.78 (s, 3H each, CH$_3$), 0.72 (d, 1H, J = 11.3 Hz), 0.60 (s, 3H, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ 176.51, 142.63, 136.08, 128.46, 128.07, 128.05, 123.01, 78.97, 75.00, 66.33, 55.30, 48.84, 46.74, 46.38, 41.36, 40.74, 39.53, 38.76, 38.54, 37.02, 35.54, 35.46, 32.94, 32.77, 30.58, 30.39, 28.09, 27.23, 27.01, 24.70, 23.31, 18.29, 17.00, 15.59, 15.46.

2. Synthesis of benzyl (4a*R*,6a*S*,6b*R*,12a*R*)-2,2,6a,6b,9,9,12a-heptamethyl-5,10 -dioxo-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a (2*H*)-carboxylate (**2**).

Pyridinium chlorochromate (PCC) reagent (590 mg, 2.75 mmol) was added to a solution of compound **1** (500 mg, 0.9 mmol) in 30 mL of CH$_2$Cl$_2$. The reaction mixture was stirred at room temperature overnight. After completion (TLC), the residue was purified by chromatography over silica gel to afford compound **2** as a white solid (390 mg, 78% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.27-7.31 (m, 5H), 5.49 (t, 1H, J = 3.6 Hz), 5.03-5.11 (m, 2H), 3.32 (dd, 1H, J = 3.7, 14.4 Hz), 2.46-2.58 (m, 2H), 2.31-2.37 (m, 1H), 2.23-2.26 (m, 1H), 1.83-1.96 (m, 4H), 1.14, 1.05, 1.01, 0.99, 0.89, 0.84, 0.64 (s, 3H each, CH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ 217.06, 208.17, 172.36, 140.27, 135.16, 128.44, 128.31, 128.28, 124.04, 66.80, 58.87, 55.04, 47.66, 47.22, 46.38, 46.15, 45.58, 45.16, 39.48, 38.89, 36.50, 34.38, 33.91, 32.66, 31.98, 30.45, 27.01, 26.61, 26.42, 23.38, 23.16, 21.35, 19.32, 16.62, 14.88; ESI-HRMS calcd for C$_{37}$H$_{50}$NaO$_4$ [M+Na]$^+$: 581.3601, found 581.3608.

3. Synthesis of (6a*R*,6b*S*,14b*R*)-4,4,6a,6b,11,11,14b-heptamethyl-1,4a,5,6,6a,6b,7,8a, 9,10,11,12,12a,14,14a,14b-hexadecahydropicene-3,8(2*H*,4*H*)-dione (**Y0**).

Compound **2** (200 mg, 0.36 mmol) was dissolved in THF/MeOH (1:1 *v/v,* 12 mL) and 10% palladium-carbon (20 mg) was added. The reaction mixture was stirred

under hydrogen gas (0.35 MPa) overnight. After removing the catalyst by filtration with the aid of celite, the filtrate was concentrated and then purified by column chromatography over silica gel to afford compound **Y0** as a white solid (125 mg, 82% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.46 (t, 1H, J = 3.6 Hz), 2.86-2.92 (m, 1H), 2.49-2.57 (m, 3H), 2.34-2.41 (m, 1H), 1.85-2.02 (m, 5H), 1.18, 1.09, 1.06, 1.05, 0.98, 0.89, 0.85 (s, 3H each, CH$_3$) (**Supplementary Fig. 14**); ^{13}C NMR (100 MHz, CDCl$_3$): δ 217.24, 214.20, 142.61, 122.33, 55.20, 47.52, 27.33, 47.11, 46.73, 46.48, 46.04, 44.73, 39.44, 38.98, 36.76, 34.33, 34.01, 33.24, 32.25, 30.89, 26.72, 26.55, 23.39, 21.41, 20.84, 19.51, 17.37, 15.02 (**Supplementary Fig. 15**); ESI-HRMS calcd for C$_{29}$H$_{45}$O$_2$ [M+H]$^+$: 425.3414, found 425.3413 (**Supplementary Fig. 16**).

Synthesis of probe Y20 (**Supplementary Scheme 4**)

1. Synthesis of 3-(3-methyl-3*H*-diazirin-3-yl)propan-1-ol (**1**).

5-hydroxy-2-pentanone (10 g, 0.1 mol) was added to 40 mL of NH$_3$ and stirred for 3 h at -78 ℃. Hydroxylamine-O-sulfonic acid (15 g, 0.13 mol) was dissolved in methanol and poured into the reaction mixture. After stirring overnight, the white precipitate was filtered and methanol was added to the ice-cold reaction mixture, followed by triethylamine. Iodine was slowly added until the iodine colour persisted. After 2 h, the methanol was evaporated and the reaction mixture was extracted with ether and dried over MgSO$_4$. Vacuum distillation was performed to obtain the final pale yellow oil **1** (4.5 g, 40.3% overall yield).

2. Synthesis of 3-(3-methyl-3*H*-diazirin-3-yl)propyl 4-methylbenzenesulfonate (**2**).

Three millilitres of triethylamine was added to a solution of compound **1** (1.14 g, 10 mmol) in 40 mL of CH$_2$Cl$_2$ at 0 ℃, followed by the step-wise addition of *p*-toluenesulfonyl chloride (2.86 g, 15 mmol). The reaction mixture was stirred at room temperature (RT) overnight. After completion, as determined by TLC, the reaction mixture was treated with 1 N HCl. The organic phase was separated and the aqueous phase was extracted with CH$_2$Cl$_2$. The combined extracts were washed with 1 N HCl and 1 N NaOH, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (petroleum ether : EtOAc, 10:1 v/v)

to yield compound **2** as a viscous oil (2.2 g, 82% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 0.97 (s, 3H), 1.38-1.42 (m, 2H), 1.49-1.56 (m, 2H), 2.45 (s, 3H), 3.99 (t, 2H, $J = 6.1$ Hz), 7.36 (d, 2H, $J = 8.0$ Hz), 7.78 (d, 2H, $J = 8.3$ Hz). ^{13}C NMR (400 MHz, CDCl$_3$): δ 19.6, 21.6, 23.5, 25.0, 30.2, 69.3, 127.8 (2C), 129.8 (2C), 132.9, 144.8.

3. Synthesis of methyl (3-(3-methyl-3*H*-diazirin-3-yl)-propyl)-glycinate (**3**).

Compound **2** (570 mg, 2.12 mmol) and methyl glycinate (568 mg, 6.37 mmol) were dissolved in 50 mL of MeCN; then, K$_2$CO$_3$ (880 mg, 6.37 mmol) and KI (1057 mg, 6.37 mmol) were added to the mixture. The mixture was refluxed at 70 ℃ overnight. The residue was purified by silica gel column chromatography (DCM:MeOH, 8:1 v/v) to afford compound **3** as a white solid with a 36% yield. Rf = 0.53 (DCM:MeOH, 8:1 v/v). ^{1}H NMR (400 MHz, CDCl3): 3.72 (s, 3H), 3.38 (s, 2H), 2.56 (t, J = 6.6 Hz, 2H), 1.36 (m, 4H), 0.99 (s, 3H); ^{13}C NMR (100 MHz, CDCl3): δ 172.86, 51.75, 50.61, 48.78, 31.89, 25.59, 24.51, 19.76. ESI-HRMS (m/z) Calcd for C$_8$H$_{15}$N$_3$O$_2$ [M+H]$^{+}$: 186.1243. Found 186.1240.

4. Synthesis of Methyl N-[3β-Hydroxy-lup-20(29)-en-28-oyl]-8-aminooctanoate (**4**).

BA OBT (166 mg, 0.28 mmol) was dissolved in 10 mL of DMF and then added to methyl 8-aminooctanoate (173 mg, 0.56 mmol) and NaCO$_3$ (100 mg, 0.94 mmol). The reaction mixture was subjected to stirring overnight. Subsequently, the reaction mixture was evaporated under a vacuum to remove the DMF; 50 mL saline was then added, and the water layer was washed with EtOAc three times, dried with Na$_2$SO$_4$, filtered and concentrated. The residue was purified by silica gel column chromatography to afford compound **4** as a white solid with a 41% yield. R$_f$ = 0.77 (petroleum ether:ethyl acetate, 3:1 v/v)

5. Synthesis of N-[3β-Hydroxy-lup-20(29)-en-28-oyl]-8-aminooctanoic acid (**5**).

Compound **4** (160 mg, 0.26 mmol) was dissolved in THF (16 mL) and MeOH (4 mL); then, an aqueous 1 N NaOH solution (1.6 mL) was added to the resulting solution at 0 ℃. The reaction mixture was stirred at room temperature for 3 h, vacuum-evaporated to remove the solvent and incubated in with an aqueous 1 N HCl

solution in an ice bath to reach a pH value of 2-3. The precipitate was collected and vacuum-dried to obtain compound **5** as a white solid, with a yield of 70%. $R_f = 0.48$. (DCM: MeOH, 10:1 v/v). ^{1}H NMR (400 MHz CDCl$_3$): δ 5.63 (t, J = 4.6 Hz, 1H), 4.71 (s, 1H), 4.57 (s, 1H), 3.37 (m, 1H), 3.22-3.05 (m, 3H), 2.42 (m, 1H), 2.32 (t, 2H, J = 7.4 Hz), 1.66 (s, 3H), 0.95, 0.92 (s, 3H each, 2 × CH$_3$), 0.88, 0.80, 0.74 (s, 3H each, 3 × CH$_3$); ^{13}C NMR (100 MHz CDCl$_3$): δ 178.77, 176.08, 150.95, 109.27, 79.02, 77.32, 77.00, 76.68, 55.58, 55.36, 50.60, 50.10, 46.73, 42.45, 40.72, 39.13, 38.80, 38.69, 38.45, 37.71, 37.16, 34.38, 33.93, 33.84, 30.86, 29.72, 29.40, 28.93, 28.83, 27.94, 27.29, 26.72, 25.60, 24.55, 20.90, 19.47, 18.25, 16.11, 16.10, 15.33, 14.61.

6. Synthesis of methyl N-(8-(((1*R*,3a*S*,5a*R*,5b*R*,9*S*,11a*R*)-9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1*H*-cyclopenta[a]chrysene-3a-carboxamido)octanoyl)-N-(3-(3-methyl-3*H*-diazirin-3-yl)propyl)glycinate (**6**).

Compound **3** (15.5 mg, 0.08 mmol) and compound **5** (50 mg, 0.08 mmol) were dissolved in 15 mL of THF and then EDC was added (14 mg, 0.13 mmol). The reaction mixture was refluxed at 50 ℃ for 12 h and then vacuum-evaporated to remove the solvent. The residue was purified by silica gel column chromatography (elute: petroleum ether:ethyl acetate = 2:1) to afford compound **6** as a white solid with a 52% yield. $R_f = 0.30$ (petroleum ether:ethyl acetate, 1:1 v/v). ^{1}H NMR (400 MHz CDCl$_3$): δ 5.62 (s, 1H), 4.71 (s, 1H), 4.57 (s, 1H), 3.98 (s, 2H), 3.76 (s, 1H), 3.70 (s, 2H), 3.26 (m, 3H), 3.14 (m, 3H), 2.44 (t, J = 11.0 Hz, 1H), 1.66 (s, 1H), 1.34 (s, 1H), 1.32 (s, 1H), 1.02 (s, 1H), 0.95 (s, 1H), 0.92 (s, 1H), 0.80 (s, 1H), 0.74 (s, 1H); ^{13}C NMR (100 MHz CDCl$_3$): δ 175.90, 173.33, 169.86, 151.03, 109.23, 78.92, 77.32, 77.00, 76.68, 55.54, 55.36, 52.05, 50.61, 50.12, 48.32, 47.55, 46.74, 42.44, 40.72, 39.11, 38.82, 38.69, 38.43, 37.69, 37.17, 34.38, 33.82, 32.55, 31.53, 31.06, 30.87, 29.72, 29.39, 29.20, 29.02, 27.95, 27.39, 26.76, 25.60, 25.19, 24.93, 23.16, 20.90, 19.93, 19.46, 18.27, 16.11, 15.33, 14.60. ESI-HRMS (m/z) Calcd for C$_{46}$H$_{76}$N$_4$O$_5$ [M+H]$^+$: 765.5895. Found 765.5905.

7. Synthesis of

N-(8-((1*R*,3a*S*,5a*R*,5b*R*,9*S*,11a*R*)-9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-1*H*-cyclopenta[a]chrysene-3a-carboxamido)octanoyl)-N-(3-(3-methyl-3*H*-diazirin-3-yl)propyl)glycine (**Y20**).

Compound **6** (12 mg, 0.016 mmol) was dissolved in 4:1 THF:MeOH; then, an aqueous 1 N NaOH solution (100 µL) was added to the resulting solution at 0 ℃. The reaction mixture was stirred at room temperature and reacted for 3 h, vacuum-evaporated to remove the solvent, and incubated with an aqueous 1 N HCl solution to reach a pH value of 2-3. The precipitate was collected and vacuum-dried to obtain **Y20** as a white solid, with a yield of 70%. ^{1}H NMR (400 MHz CDCl$_3$): δ 5.78 (m, 1H), 4.71 (s, 1H), 4.57 (s, 1H), 3.98 (s, 2H), 3.27 (m, 3H), 3.13 (m, 3H), 2.20 (t, *J* = 7.2 Hz, 1H), 1.66 (s, 1H), 1.34 (s, 1H), 1.32 (s, 1H), 1.02 (s, 1H), 0.94 (s, 1H), 0.91 (s, 1H), 0.80 (s, 1H), 0.74 (s, 1H) (**Supplementary Fig. 17**); ^{13}C NMR (100 MHz CDCl$_3$): δ 176.51, 173.99, 171.89, 151.89, 109.29, 79.02, 77.32, 77.00, 76.68, 55.58, 55.32, 52.05, 50.57, 50.07, 48.61, 47.01, 46.73, 42.44, 40.71, 39.21, 38.79, 38.67, 38.47, 37.70, 37.14, 34.35, 33.76, 32.55, 31.58, 31.03, 30.84, 29.60, 29.38, 29.06, 28.87, 27.94, 27.26, 26.70, 25.58, 25.20, 24.96, 23.10, 20.89, 19.90, 19.44, 18.26, 16.12, 15.36, 14.61 (**Supplementary Fig. 18**). ESI-HRMS (m/z) Calcd for C$_{45}$H$_{74}$N$_4$O$_5$ [M+H]$^+$: 751.5738. Found 751.5732 (**Supplementary Fig. 19**).

Synthesis of probe Y21 (**Supplementary Scheme 5**)

1. Synthesis of 3-methyl-3*H*-diazirine-3-propanoic acid (**1**).

    A solution of levulinic acid (500 mg, 4.3 mmol) in MeOH (2.0 mL) was cooled in an ice bath. To this solution was added 7 N ammonia (5.0 mL in MeOH) via syringe. The mixture was allowed to stir for 3 h at 0 ℃ at which point a solution of hydroxylamine-O-sulfonic acid (729 mg, 6.5 mmol) in MeOH (5 mL) was added. The reaction mixture was stirred for 16 h while the ice-water bath was allowed to warm to 25 ℃. The excess ammonia was removed by gently blowing air through the suspension for 1 h using a glass pipette. The suspension was filtered and the filtrate concentrated by rotary evaporation. The solid mass was dissolved MeOH (20 mL) and cooled in an ice bath. Triethylamine (1.1 mL, 7.9 mmol) was added and the solution

was allowed to stir for 5 minutes. Iodine was added until the color remained. The solution was diluted with EtOAc (30 mL) and successively washed with 1 N HCl (20 mL), a 10% sodium thiosulfate solution (20 mL), saturated aqueous NaCl (20 mL), and finally dried over anhydrous Na_2SO_4. The organic solvents were removed by rotary evaporation to afford yellow oil **1** (375 mg, 68% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 11.54 (s, 1H), 2.25 (t, 2H, J = 7.6 Hz), 1.73 (t, 2H, J = 7.6 Hz), 1.05 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$): δ 178.83, 29.22, 28.48, 25.00, 19.58.

2. Synthesis of 3-methyl-3*H*-diazirine-3-propanoyl chloride (**2**).

　　Oxalyl chloride (471 μL, 5.6 mmol) and DMF (4 drops) were added to a solution of **1** (178 mg, 1.4 mmol) in 12 mL of redistilled DCM at 0℃. The reaction mixture was stirred at room temperature (RT) overnight. After completion, the solvents were removed by rotary evaporation to give **2** without further purification.

3. Synthesis of β-D-galactopyranosyl azide (**3**).

　　To a stirring solution of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl azide (1.7 g, 4.6 mmol) in dry MeOH (30 mL) was added a solution of sodium methoxide (1M in MeOH) until pH 9-10 (250 μL). The reaction mixture was stirred at r.t. for 3 h. The solution was then neutralized by addition of ionexchange resin (Dowex 50WX4) until pH 7, filtered, and the solvent was removed under reduced pressure. The residue was then purified by column chromatography (CH$_2$Cl$_2$:CH$_3$OH, 8:1 v/v) to give **3** as a white solid (820 mg, 4.0 mmol, 87% yield).

4. Synthesis of 6-O-(3-methyl-3*H*-diazirine-3-propionyl)-β-D-galactopyranosyl azide (**4**).

　　To a stirring solution of **3** (212 mg, 1.03 mmol) in DMF/THF (20/15 mL) was added a solution of **2** (~1.2 mmol) in DCM (8 mL) and TEA (428 μL, 3.09 mmol) at 0 ℃. The reaction mixture was stirred at RT overnight. After completion, as determined by TLC, the reaction mixture was concentrated under reduced pressure. The residue was then purified by column chromatography (CH$_2$Cl$_2$:CH$_3$OH, 30:1 v/v) to give **4** as the light brown oil (114 mg, 0.36 mmol, 35% yield). ^{1}H NMR (400 MHz,

CDCl$_3$): δ 4.30-4.77 (m, 6H), 4.00 (s, 1H), 3.85 (t, 1H, J = 4.9 Hz), 3.59-3.69 (m, 2H), 2.25 (t, 2H, J = 7.5 Hz), 1.74 (t, 2H, J = 7.5 Hz), 1.05 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$): δ 172.59, 90.66, 74.54, 73.30, 70.66, 68.86, 63.45, 29.44, 28.69, 25.24, 19.56.

5. Synthesis of 2,3,4-tri-O-acetyl-6-O-(3-methyl-3*H*-diazirine-3-propionyl)-β-D-galactopyranosyl azide (**5**).

To a solution of **4** (100 mg, 0.32 mmol) in anhydrous pyridine (5 mL) at 0 ℃ acetic anhydride (0.44 mL) was added, and the solution was stirred overnight at RT. The solution was moved under reduced pressure and then diluted with dichloromethane, and the organic phase was dried with Na$_2$SO$_4$. The residue was then purified by column chromatography (petroleum ether:EtOAc, 4:1 v/v) to give **5** as the colorless oil (136 mg, 0.31 mmol, 97% yield). ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.39 (d, 1H, J = 2.0 Hz), 5.12 (t, 1H, J = 9.6 Hz), 5.02 (dd, 1H, J = 10.3, 2.8 Hz), 4.60 (d, 1H, J = 8.6 Hz), 4.16 (m, 2H), 4.03 (t, 1H, J = 6.4 Hz), 2.17 (t, 2H, J = 7.5 Hz), 2.14 (s, 3H), 2.06 (s, 3H), 1.95 (s, 3H), 1.67 (t, 2H, J = 7.5 Hz), 1.00 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$): δ 171.57, 169.99, 169.82, 169.24, 88.20, 72.71, 70.63, 68.03, 66.82, 61.38, 29.42, 28.48, 24.99, 20.54, 20.48, 20.39, 19.46. ESI-HRMS (m/z) Calcd for C$_{17}$H$_{23}$N$_5$O$_9$ [M+Na]$^+$: 464.1388. Found 464.1377.

6. Synthesis of 2,3,4-tri-O-acetyl-6-O-(3-methyl-3*H*-diazirine-3-propionyl)-β-D-galactopyranosyl amide (**6**).

To a solution of **5** (116 mg, 0.26 mmol) in THF/H$_2$O (4/4 mL) triphenylphosphine (206 mg, 0.79 mmol) was added, and the solution was stirred overnight at r.t. After completion, the solvents were removed by rotary evaporation to give **6** without further purification.

7. Synthesis of (4a*R*, 5*R*, 6a*S*, 6b*R*, 10*S*, 12a*R*)-10-hydroxy-2,2,6a,6b,9,9,12a-heptamethyl-2,3,4-tri-O-acetyl-6-O-(3-methyl-3*H*-diazirine-3-propionyl)-β-D-galactopyranosyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2*H*)-carboxamide (**Y21**).

N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, 108 mg, 0.54 mmol) was added to oleanolic acid (OA, 88 mg, 0.19 mmol) with stirring in 6 mL of dry THF. After 0.5 h, **6** (~0.26 mmol) was added to the mixture. The mixture was stirred at RT until completion, as determined by TLC. The solvent was evaporated, the residue was suspended with EtOAc (30 mL), and washed with water (20 mL $\times$ 3) and brine, and dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (petroleum ether:EtOAc, 2:1 v/v) to give **Y21** as a white solid (114 mg, 0.13 mmol, 68% yield). ^{1}H NMR (400 MHz, CDCl₃): δ 6.65 (d, 1H, J = 8.9 Hz), 5.48 (t, 1H, J = 3.0 Hz), 5.38 (d, 1H, J = 2.8 Hz), 5.16-5.00 (m, 3H), 4.10-3.94 (m, 3H), 3.20 (dd, 1H, J = 10.2, 2.4 Hz), 2.53 (dd, 1H, J = 12.8, 2.9 Hz), 2.15 (t, 2H, J = 7.6 Hz), 2.11 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.84-1.16 (m, 24H), 1.14 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H), 0.78 (s, 3H), 0.77 (s, 3H), 0.71 (m, 1H) (**Supplementary Fig. 20**); ^{13}C NMR (100 MHz, CDCl₃): δ 178.92, 171.59, 171.07, 169.95, 169.71, 143.70, 123.46, 78.88, 78.61, 71.69, 70.74, 68.27, 67.06, 60.93, 55.12, 47.51, 46.57, 46.42, 41.97, 41.29, 39.34, 38.72, 38.51, 36.92, 34.13, 32.83, 32.51, 30.58, 29.45, 28.48, 28.04, 27.21, 27.13, 25.47, 24.99, 24.06, 23.52, 23.19, 20.74, 20.52, 20.50, 19.50, 18.27, 17.06, 15.52, 15.34 (**Supplementary Fig. 21**). ESI-HRMS (m/z) Calcd for $C_{47}H_{71}N_3O_{11}$ [M+H]$^+$: 854.5161. Found 854.5159 (**Supplementary Fig. 22**).

Synthesis of compounds Y1-Y4, Y9-Y16 and Y19.

The synthesis procedures and characterization of compounds **Y1**-**Y4** and **Y9**-**Y16** have been reported in our previous study[1]. And the synthesis procedure and characterization of compound **Y19** have been reported by other group[3].

Supplementary Scheme 1. Synthesis of probe Y18.
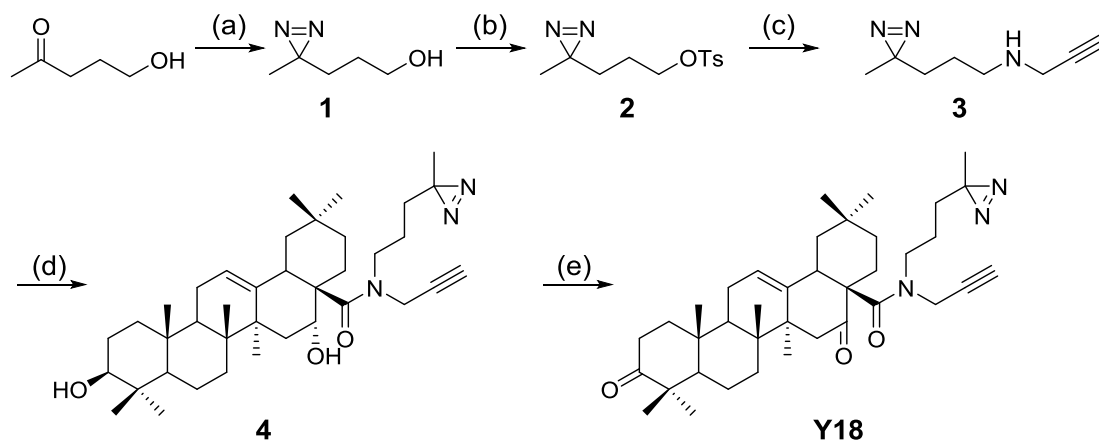
(a) NH$_3$, Hydroxylamine-O-sulfonic acid, I$_2$, TEA, -40 ℃ to RT overnight;

(b) TsCl, TEA, CH$_2$Cl$_2$, 0 ℃ to RT, overnight;

(c) Propargyl bromide, K$_2$CO$_3$, Acetone, reflux, overnight;

(d) EA, EDC, THF, RT, overnight;

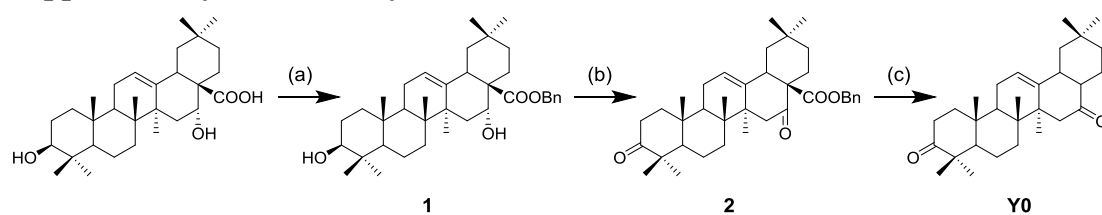(e) Dess-Martin periodinane, CH$_2$Cl$_2$, RT, 30 min.

Supplementary Scheme 2. Synthesis of Biotin-azide.

1

Biotin-azide
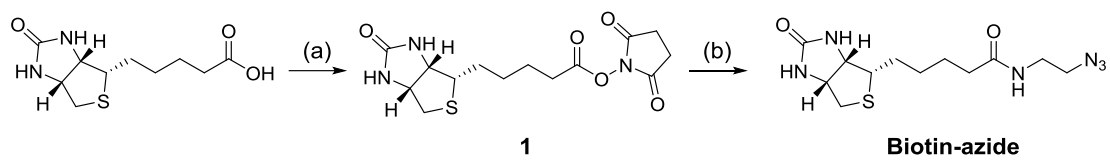
(a) N-Hydroxysuccinimide, EDC, DMF, RT;

(b) 2-azidoethylamine, TEA, DMF, RT.

Supplementary Scheme 3. Synthesis of Y0.

(a) Benzyl bromide, K_2CO_3, DMF, 60 °C, 24 h;

(b) PCC, CH_2Cl_2, RT, overnight;

(c) H_2, Pd/C, THF/MeOH, RT, overnight.

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Supplementary Scheme 4. Synthesis of probe Y20.

(a) liquid ammonia, NH_2OSO_3H, I_2, methanol, trimethylamine, RT, 5 h;

(b) TsCl, pyridine, overnight;

(c) methyl glycinate, KI, K_2CO_3, CH_3CN, 70 ℃, overnight;

(d) methyl 8-amino-octanoate, Na_2CO_3, DMF, overnight;

(e) NaOH (1 N), THF, methanol, RT, 3 h;

(f) EDC, THF, 50 ℃, RT, refluxed;

(g) NaOH (1 N), THF, methanol, RT, 3 h.

Supplementary Scheme 5. Synthesis of probe Y21.
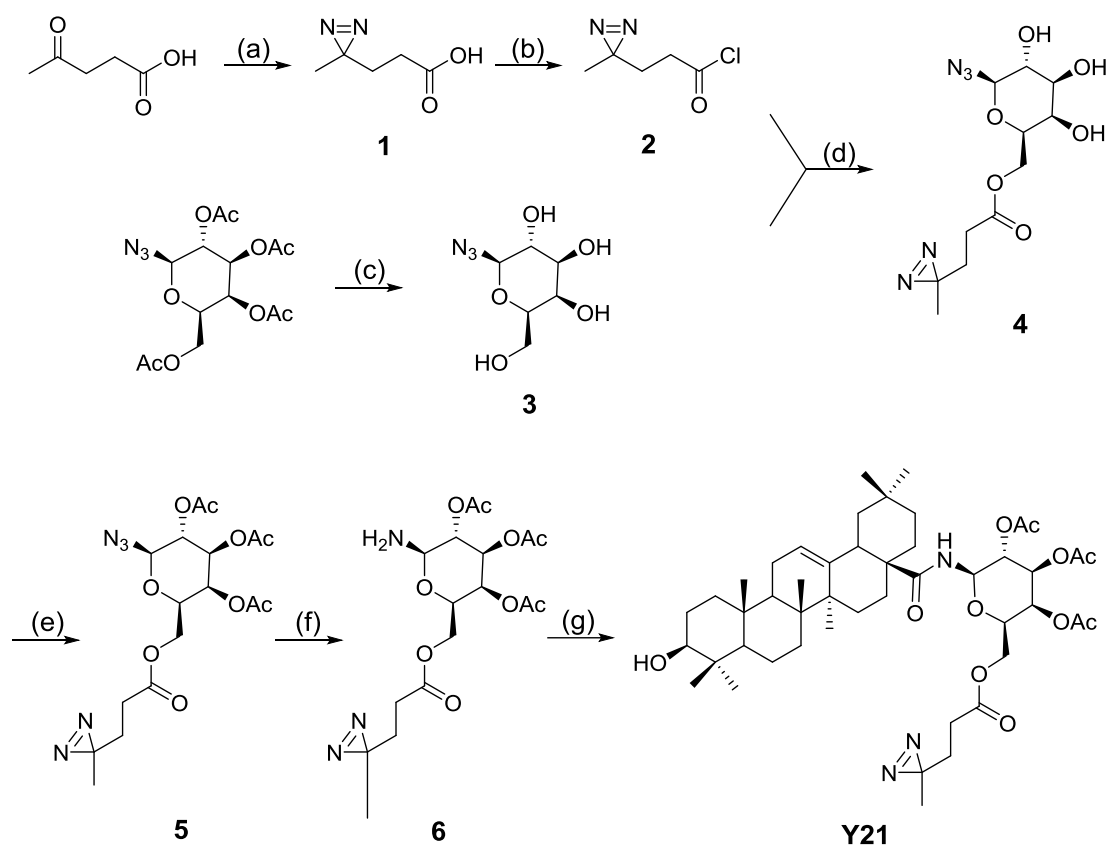
(a) NH₃, MeOH, NH₂OSO₃H, I₂, Et₃N, 0 °C to RT;

(b) (COCl)₂, DCM ᴅMF, 12 h;

(c) MeONa, MeOH, RT, 2-3 h;

(d) DMF:THF:DCM=2:1:1, TEA, 0 °C to RT, 25h;

(e) Ac₂O, pyridine, RT, 12 h;

(f) Ph₃P, THF, H₂O, RT, overnight;

(g) OA, EDC, THF, 60 °C, overnight.

Supplementary Figure 11. ¹H-NMR spectrum of Y18.

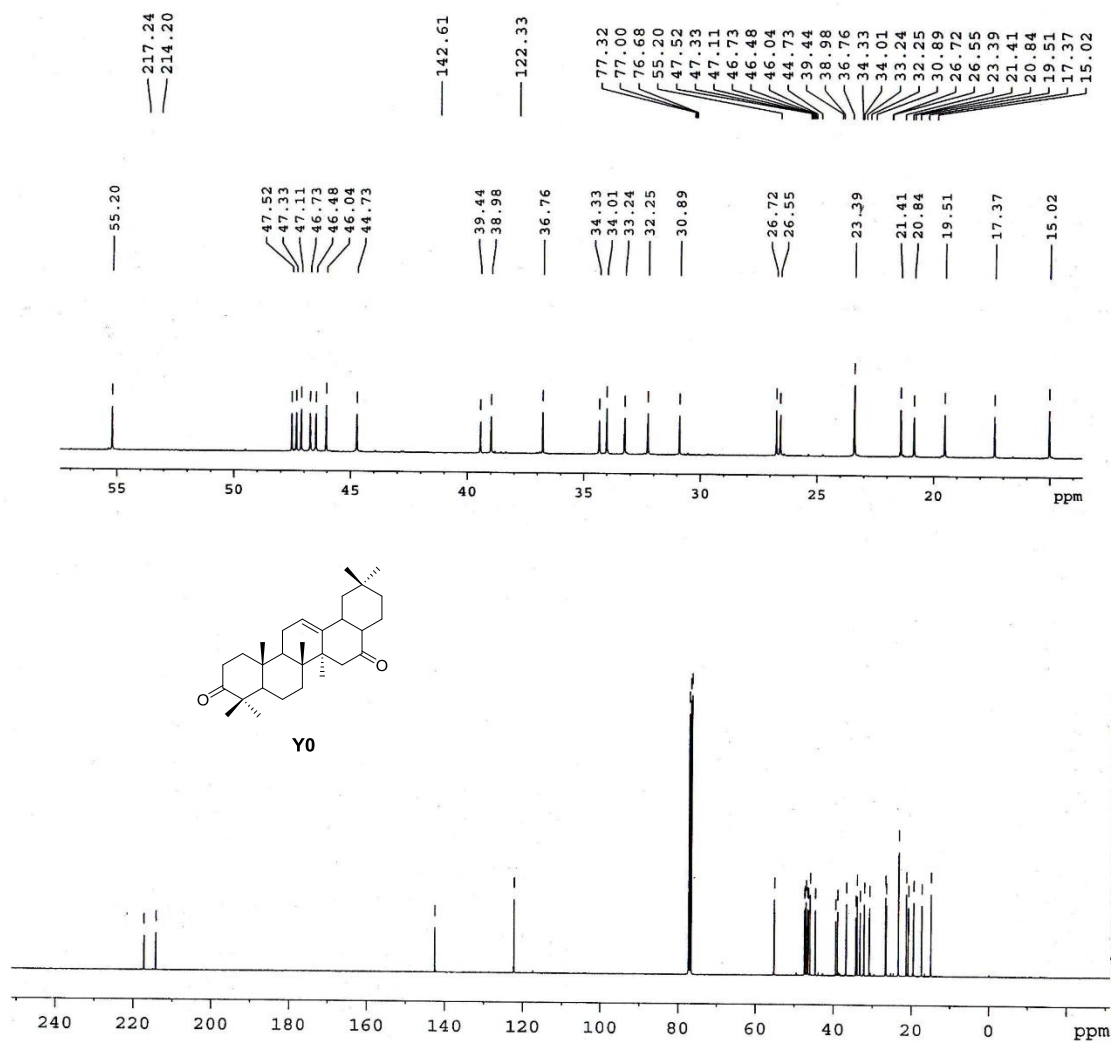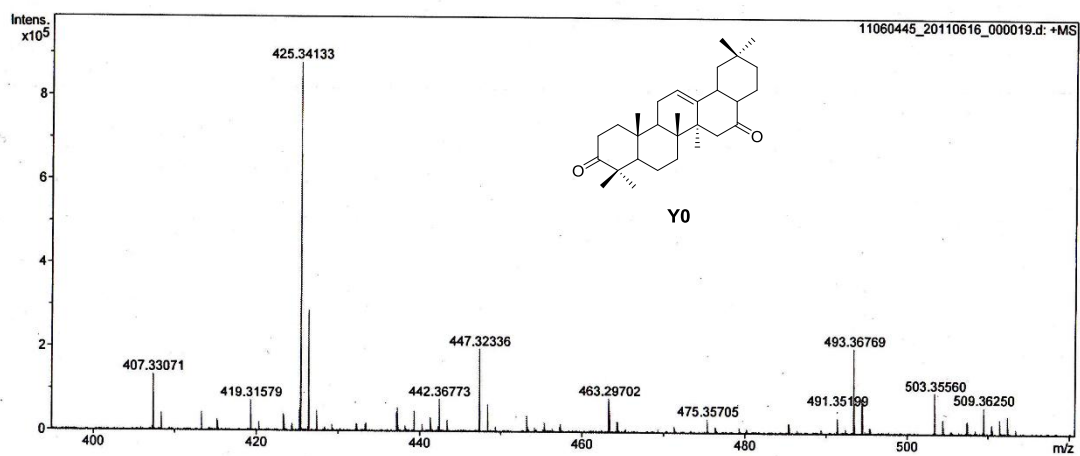
Supplementary Figure 12. ¹³C-NMR spectrum of Y18.

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Supplementary Figure 13. High resolution mass spectrum of Y18.
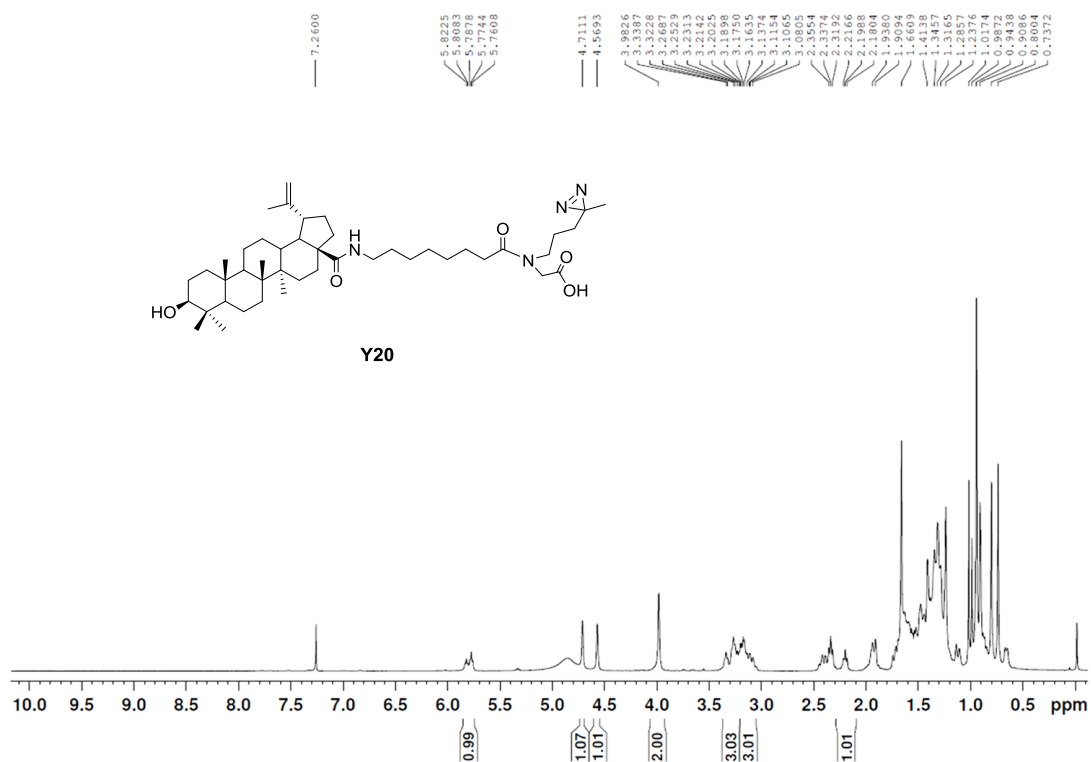

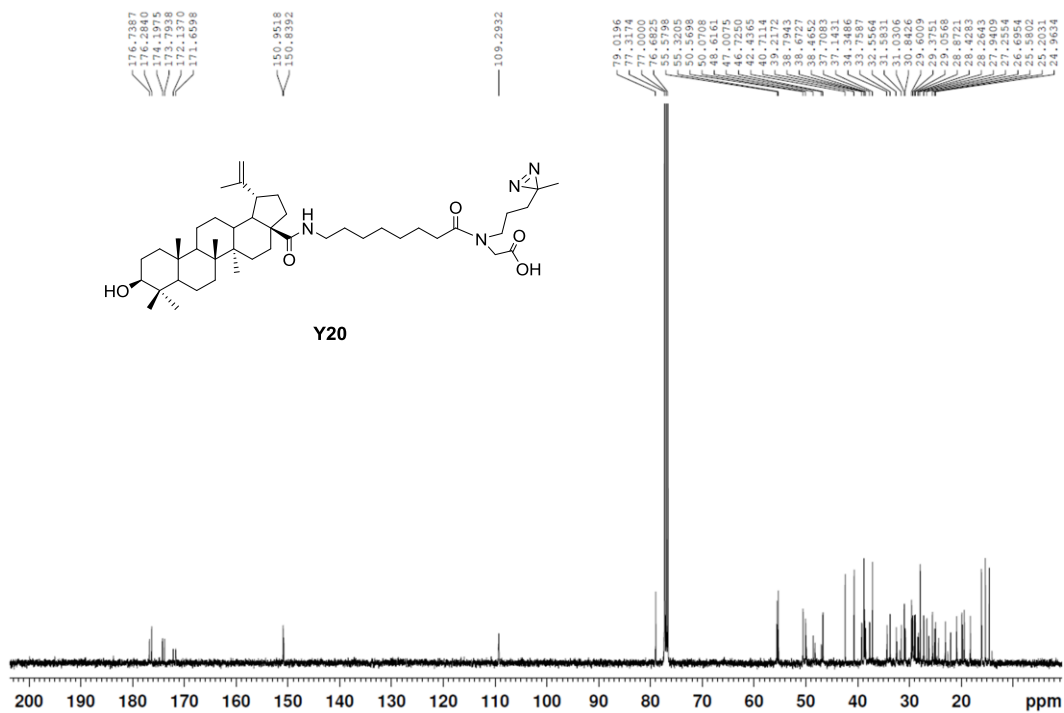**Supplementary Figure 14. ¹H-NMR spectrum of Y0.**

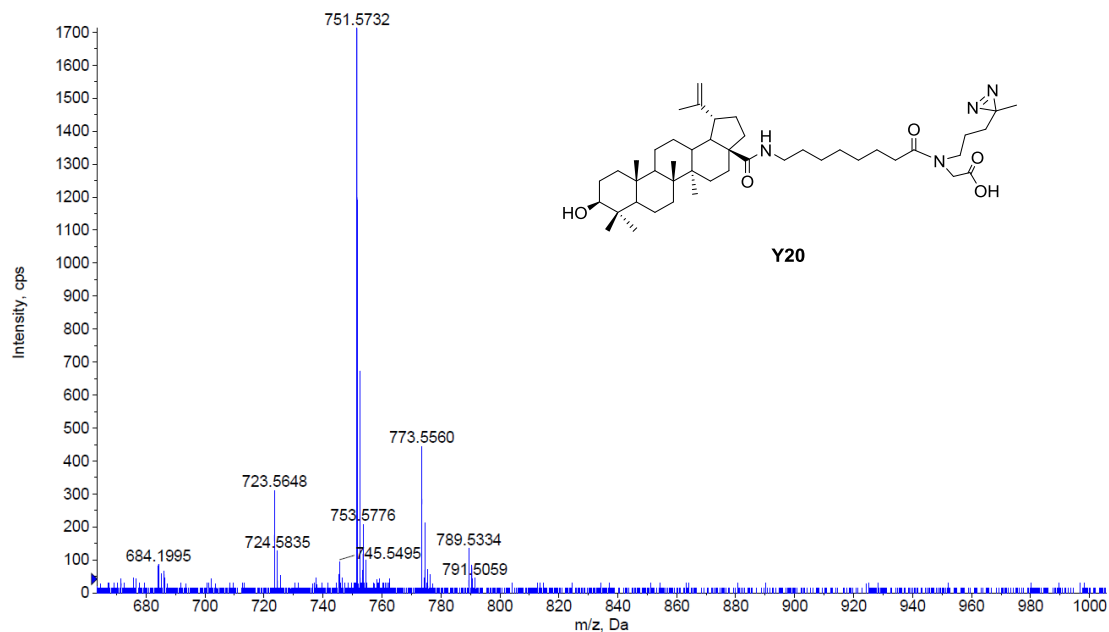**Supplementary Figure 15. ^{13}C-NMR spectrum of Y0.**

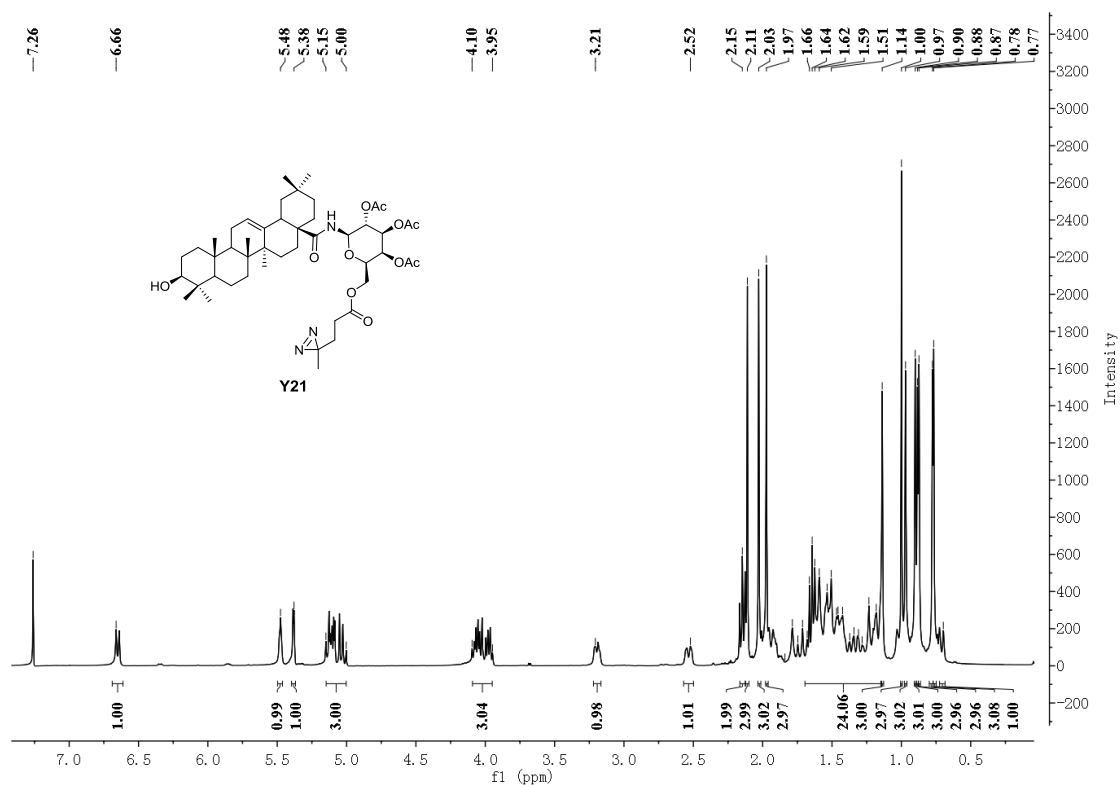**Supplementary Figure 16. High resolution mass spectrum of Y0.**
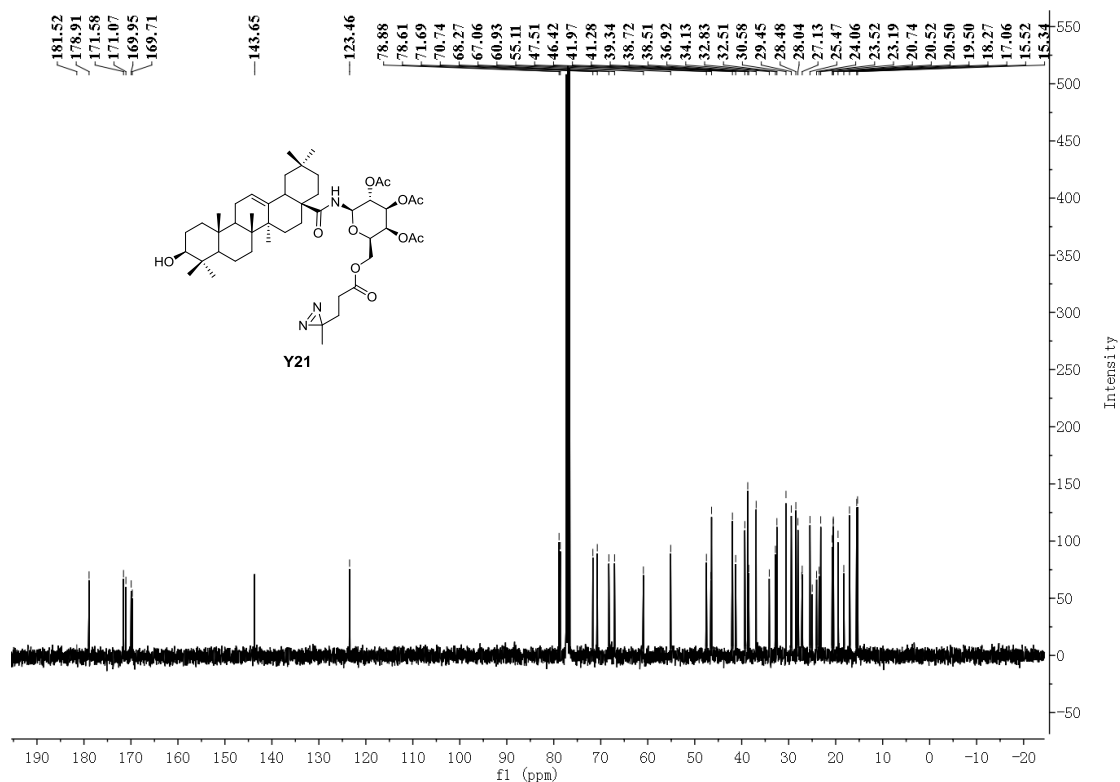
Supplementary Figure 17. ¹H-NMR spectrum of Y20.

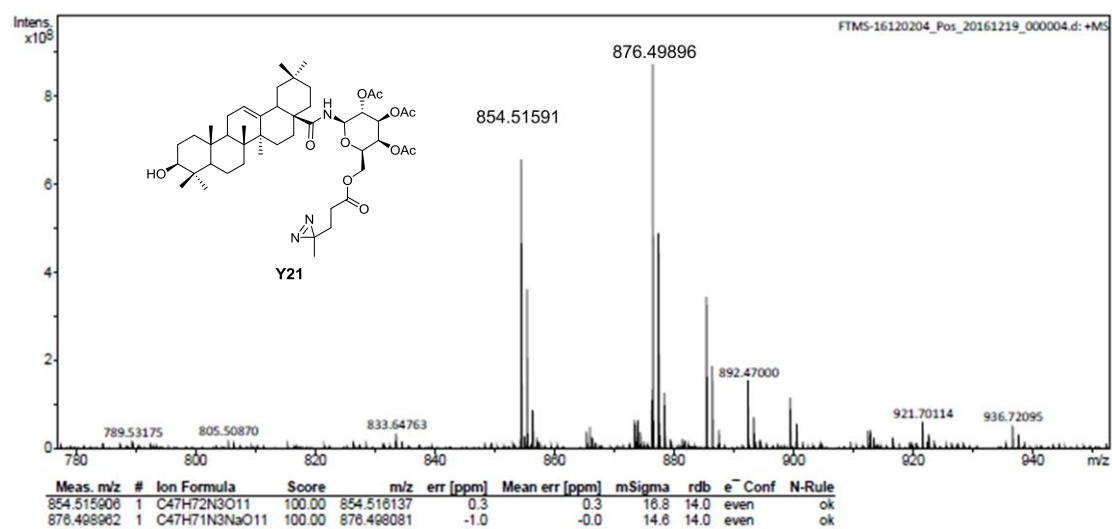**Supplementary Figure 18. ¹³C-NMR spectrum of Y20.**

Supplementary Figure 19. High resolution mass spectrum of Y20.

Supplementary Figure 20. ^{1}H-NMR spectrum of Y21.

Supplementary Figure 21. ^{13}C-NMR spectrum of Y21.

Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
854.515906	1	C47H72N3O11	100.00	854.516137	0.3	0.3	16.8	14.0	even	ok
876.498962	1	C47H71N3NaO11	100.00	876.498081	-1.0	-0.0	14.6	14.0	even	ok

Supplementary Figure 22. High resolution mass spectrum of Y21.

Supplementary References

1   Yu, M. R. *et al.* Discovery of Pentacyclic Triterpenoids as Potential Entry Inhibitors of Influenza Viruses. *J Med Chem* **57**, 10058-10071 (2014).

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3   Soler, F. *et al.* Betulinic acid derivatives: A new class of specific inhibitors of human immunodeficiency virus type 1 entry. *J Med Chem* **39**, 1069-1083 (1996).